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Breast cancer inhibiting diastereomeric diacetato[1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) derivatives: synthesis and studies on the relationship between reactivity and antitumor activity [1]

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Abstract

Antitumor active [1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) diastereoisomers containing acetic acid derivatives as 'leaving groups' (acetate: *meso/rac*-4F-Pt(Ac)$_2$; monochloroacetate: *meso/rac*-4F-Pt(ClAc)$_2$; dichloroacetate: *meso/rac*-4F-Pt(Cl$_2$Ac)$_2$; trichloroacetate: *meso/rac*-4F-Pt(Cl$_3$Ac)$_2$; glycolate: *meso/rac*-4F-Pt(OHAc)$_2$; phenylacetate: *meso/rac*-4F-Pt(PhAc)$_2$) were synthesized and characterized by IR and ^{1}H NMR spectroscopy. In all complexes except *meso/rac*-4F-Pt(PhAc)$_2$, which exist as [*meso/rac*-4F-PtPhAc]$^+$PhAc$^-$, both carboxylic acid residues are coordinated to platinum. Kinetic studies on the reaction behavior of the title compounds with nucleophiles were performed by using iodide as nucleophile. The studies show that the new complexes react with nucleophiles predominantly via the 'solvent path' (i.e. via the reactive intermediates =Pt(X)(OH$_2$)$^+$ and =Pt(OH$_2$)$_2^{2+}$). Therefore the rates of the reactions in which the reactive species are formed affect the antitumor activity of the complexes as well as their inactivation by bionucleophiles during the transport to the tumor. The extent of accumulation in the tumor cell, too, influences the antitumor activity of a complex. The rate constants are discussed in view of the activities of the respective complexes on the human MCF-7 breast cancer cell line. From the title compounds the Cl$_2$Ac and Cl$_3$Ac derivatives do not come close to the standard cisplatin, neither in chemical reactivity nor in biological activity. *Meso/rac*-4F-Pt(Ac)$_2$ and *meso/rac*-4F-Pt(ClAc)$_2$, respectively, show similar hydrolysis rates but lower antitumor activities than cisplatin, presumably due to a reduced drug uptake by the tumor cell. *Meso/rac*-4F-Pt(PhAc)$_2$ compare well with their standard carboplatin in respect to both properties. Other than the remaining, poorly water soluble title compounds, *meso/rac*-4F-Pt(OHAc)$_2$ equal their standard cisplatin in terms of water solubility and antitumor activity (*rac*-4F-Pt(OHAc)$_2$ > *meso*-4F-Pt(OHAc)$_2$). However, they are markedly faster hydrolyzed than cisplatin. By use of *rac*-4F-Pt(Ac)$_2$ as an example it was confirmed that, in contrast to the parent compound *rac*-4F-PtCl$_2$, the new complex type is also active under in vivo conditions owing to its markedly lower reactivity (mainly due to the lack of a direct substitution by strong nucleophiles), which entails a reduced inactivation of the drug on its way to the tumor. The in vitro testing on tumor cell lines combined with the evaluation of the water solubility and with kinetic studies on the reaction with nucleophiles is a useful method for the preselection of potent platinum complexes deserving further thorough in vitro and in vivo investigations.

Keywords: Antitumor activity; Platinum complexes; Chelating diamine complexes; Acetato complexes; Diastereomeric complexes

1. Introduction

Comparative studies on the cytotoxic effect of cisplatin [2] in malignant and in normal cells indicate a definite though small tumor cell selectivity of this drug [5]. The correlation of cytotoxicity with the number of DNA bound cisplatin molecules seen in these experiments points to an identical mechanism of action in malignant and in normal cells. As the main reason for the tumor specific activity of cisplatin a differently efficient DNA repair in tumor cells and in normal cells is discussed [5]. In addition, changes in the levels of glutathione and metallothionein, which inactivate cisplatin, and a reduced cisplatin accumulation in normal cells may also contribute to the observed tumor selectivity of cisplatin, e.g. in testicular cancer, which can be cured by multichemotherapy containing cisplatin as an indispensable component [6]. With the intention of enhancing the insufficient thera-

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[1] Dedicated to Professor Dr Dres. h. c. Herbert Oelschläger on the occasion of his 75th birthday.

[2] For literature concerning detection of cisplatin see Ref. [1]; application in therapy see Ref. [2]; mode of action see Refs. [3,4].

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peutic index of cisplatin numerous 'leaving group' derivatives of the *cis*-diammineplatinum(II) type were synthesized and tested [2,7]. None of these 'second generation platinum complexes' proved to be more antitumor active than cisplatin [7]. Carboplatin, for instance, the therapeutically most important 'second generation platinum complex', which is derived from cisplatin by exchange of the chloride 'leaving groups' by the more strongly bound, bidentate cyclobutane-1,1-dicarboxylate residue (CBDCA), produces an equally strong antitumor effect like cisplatin, but at a markedly higher dosage due to the delayed formation of the active aquated metabolites, $(NH_3)_2Pt(CBDCA)(OH_2)$ and $(NH_3)_2Pt(OH_2)_2^{2+}$ [5]. The therapeutic benefit of carboplatin compared to that of cisplatin is attributed to its better tolerability [7,8]. However, by comparative studies on the ADJ/PC-6 plasmacytoma of the mouse, no higher therapeutic index for carboplatin than for the parent compound cisplatin was found (MTD/ID_{90}: cisplatin = 4.1, carboplatin = 4.2; MTD: maximal tolerated dose [8]; ID_{90}: dose which produces a 90% regression of ADJ/PC-6 [5]). In addition, this result confirms that in the case of carboplatin, too, both aquated metabolites, whose formation is regulated by the hydrolysis rates, are responsible for its cytotoxicity as well as for its side effects. Therefore, the higher tolerance of 'leaving group' derivatives of cisplatin like carboplatin is invariably accompanied by a lower antitumor activity [5,8]. In vivo the antitumor activity of a platinum(II) complex is additionally influenced by its pharmacokinetic behavior. For instance, hydrolysis-sensitive compounds like nitrato- and sulfatoplatinum(II) complexes bind in a fast reaction to bionucleophiles, especially to chloride ions, and irreversibly to nucleophilic centers (especially to S-containing residues) of plasma components [9]. From the latter process insufficient free drug levels can result, which in turn lead to inadequate antitumor activities. Therapeutically suitable 'second generation platinum complexes' must have a hydrolysis behavior which guarantees an optimal free drug level in plasma as well as a cytotoxic level of aquated metabolites within the tumor cell.

Our concept for the development of platinum(II) complexes with larger therapeutic indices than cisplatin and carboplatin, respectively, aims at the search for ligands which endow the new complex with the capability to accumulate in tumor cells (i.e. 'drug targeting concept') [10]. Compounds with such properties are reckoned among the 'third generation platinum complexes'. A very promising result of our project concerns the development of the diastereomeric dichloro-[1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) complexes (*meso*- and *rac*-4F-$PtCl_2$), which are enriched in human breast cancer cells and are bound to DNA to a much greater extent than cisplatin (maximal intracellular Pt concentration (μM): *rac*-4F-$PtCl_2$ = 118.0; *meso*-4F-$PtCl_2$ = 36.2; cisplatin = 5.6) [4,9,11]. Because of these special properties we initially assumed that *meso*- and *rac*-4F-$PtCl_2$ are more effective breast cancer drugs than cisplatin [3]. We could not confirm this presumption in an experiment on the hormone-sensitive MXT-M-3.2 breast cancer of the mouse. In contrast, the diastereomeric 4F-$PtCl_2$ complexes even proved to be inactive in this model due to their insufficient bioavailabilities [15]. By the development of a colloidal drug preparation from *rac*-4F-$PtCl_2$ (i.e. *rac*-4F-$PtCl_2$ hydrosol) we were able to overcome this difficulty [4]. However, the free drug level, which was obtainable with this formulation in animal experiments, was still too low to bring about the maximal breast cancer-inhibiting effect [9]. Comparative studies on the in vitro binding kinetics to human serum albumin demonstrated a faster decrease of the free (i.e. non-albumin bound) drug level of the *rac*-4F-$PtCl_2$ formulation than of cisplatin (conditions: complex 3 μM, albumin 500 μM, temperature 37 °C; results ($t_{1/2}$ in h): cisplatin ~3, *meso*- and *rac*-4F-$PtCl_2$ formulations ~0.5) [9].

Therefore, we continued our efforts to optimize the in vivo antitumor activity of this complex type (i.e. 4F-$PtCl_2$) by:

(i) the systematic variation of the 'leaving groups'; the goal of this synthetic study is the development of complexes endowed with a reactivity against bionucleophiles which guarantees an optimal pharmacokinetic behavior and by this an optimal breast cancer-inhibiting effect in vivo [4];

(ii) the comparative testing of the new 'leaving group' derivatives and of the parent compounds on breast cancer models;

(iii) the evaluation of the water solubility of the new 'leaving group' derivatives;

(iv) the determination of the rate constants for the reaction of the new 'leaving group' derivatives with the model nucleophile I^- and their comparison with those of the parent compounds;

(v) a thorough examination of the most interesting 'leaving group' derivatives for their pharmacokinetic behavior as well as for their breast cancer-inhibiting and toxicological properties to determine their therapeutic indices.

In this publication we report on the synthesis of diastereomeric [1,2-bis(4-fluorophenyl)ethylenediamine]-platinum(II) complexes which contain acetic acid and acetic acid derivatives as 'leaving groups' and on the evaluation of their chemical and biological properties. The used 'leaving groups' are indicated in Scheme 1. The studies mentioned in (v) are in preparation and shall be described in a subsequent publication.

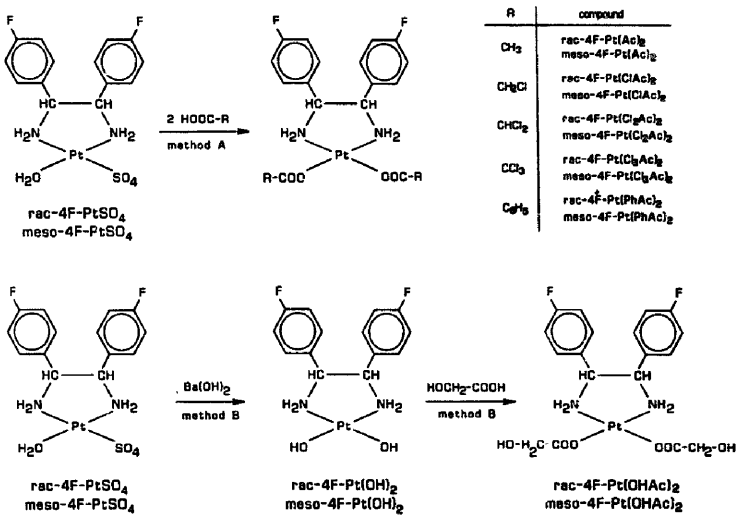
2. Experimental

2.1. General procedures

IR spectra (KBr pellets): Perkin-Elmer model 580 A. ^1H NMR of the platinum complexes: Bruker PFR-NMR spec-

[3] For the failure of cisplatin in breast cancer therapy compare Refs. [12–14].

[4] Concerning the assignment of platinum complexes to classes of differing in vivo antitumor activities due to their reactivities see Cleare et al. [16].



Scheme 1. Synthesis of the diacetatoplatinum(II) derivatives.

trometer WM 250 at 250 MHz (internal standard: TMS); the data are listed in Table 2. Elemental analyses: Microlaboratory of the University of Regensburg; based on the C, H and N analyses, all compounds were of acceptable purity (within 0.4% of the calculated values).

2.2. Materials and chemical methods

2.2.1. Chemicals

Reagents (A-grade purity) were obtained from E. Merck (Darmstadt, Germany). *N*-Hexamethylpararosaniline (crystal violet) and trypsin (1:250 vol./vol.) were purchased from Serva (Heidelberg, Germany). *N,N*-Dimethylformamide (DMF, spectrophotometric grade) was obtained from Aldrich (Steinheim, Germany). Deionized water, produced by means of a Millipore Milli-Q Water System, resistivity = 18 MΩ, was used throughout the kinetic experiments.

2.2.2. Drugs for comparative kinetic and pharmacological studies

Cisplatin was obtained from Aldrich (Steinheim, Germany); carboplatin from Sigma (Deisenhofen, Germany). *Meso*-4F-$PtCl_2$, *rac*-4F-$PtCl_2$, *meso*-4F-$PtSO_4$ and *rac*-4F-$PtSO_4$ were synthesized as previously described [17].

2.2.3. Synthesis of the diastereomeric diacetato[1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) complexes and of their 'leaving group' analogues

Method A. 1 mmol of the respective acetic acid derivative was added to a solution of 0.5 mmol 4F-$PtSO_4$ in 70 ml of water. The reaction mixture was stirred for 1 day at r.t. and the formed precipitate was sucked off. For recrystallization the complex was dissolved in a small amount of methanol and water 2:1 (vol./vol.). After standing for 48 h to allow the methanol to evaporate the chemically pure complex precipitated. It was separated by suction filtration and dried over P_2O_5 in vacuo.

Diacetato[*meso*-1,2-bis(4-fluorophenyl)ethylenediamine]-platinum(II), *meso*-4F-Pt$(Ac)_2$: white powder; yield 88%; IR (KBr): 3240 m, 3120 m (NH), 1610 m (C=O), 1230 s, 825 m. *Anal.* Calc. for $C_{18}H_{20}F_2N_2O_4Pt \cdot H_2O$: C, H, N.

Diacetato[DL-1,2-bis(4-fluorophenyl)ethylenediamine]-platinum(II), *rac*-4F-Pt$(Ac)_2$: white powder; yield 81%; IR (KBr): 3200 m, 3080 m (NH), 1610 m (C=O), 1240 s, 840 s. *Anal.* Calc. for $C_{18}H_{20}F_2N_2O_4Pt \cdot H_2O$: C, H, N.

[Bis(monochloroacetato)][*meso*-1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II), *meso*-4F-Pt$(ClAc)_2$: white powder; yield 39%; IR (KBr): 3200 m, 3080 m (NH), 1620 m (C=O), 1250 s. 830 m. *Anal.* Calc. for $C_{18}H_{18}Cl_2F_2N_2O_4Pt \cdot H_2O$: C, H, N.

[Bis(monochloroacetato)][DL-1,2-bis(4-fluorophenyl)-ethylenediamine]platinum(II), *rac*-4F-Pt$(ClAc)_2$: white powder; yield 56%; IR (KBr): 3200 m, 3120 m (NH), 1630 m (C=O), 1240 s, 840 m. *Anal.* Calc. for $C_{18}H_{18}Cl_2F_2N_2O_4Pt \cdot H_2O$: C, H, N.

[Bis(dichloroacetato)][*meso*-1,2-bis(4-fluorophenyl)-ethylenediamine]platinum(II), *meso*-4F-Pt$(Cl_2Ac)_2$: white powder; yield 90%; IR (KBr): 3200 m, 3120 m (NH), 1660 m (C=O), 1240 s, 830 m. *Anal.* Calc. for $C_{18}H_{16}Cl_4F_2N_2O_4Pt \cdot H_2O$: C, H, N.

[Bis(dichloroacetato)][DL-1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II), *rac*-4F-Pt$(Cl_2Ac)_2$: white powder; yield 92%; IR (KBr): 3200 m, 3120 m (NH), 1660 m (C=O), 1240 s, 830 m. *Anal.* Calc. for $C_{18}H_{16}Cl_4F_2N_2O_4Pt \cdot H_2O$: C, H, N.

[Bis(trichloroacetato)][*meso*-1,2-bis(4-fluorophenyl)-ethylenediamine]platinum(II), *meso*-4F-Pt$(Cl_3Ac)_2$: white powder; yield 75%; IR (KBr): 3200 m, 3110 m (NH), 1680

m (C=O), 1610 s, 1240 s, 840 m. *Anal.* Calc. for $C_{18}H_{14}Cl_6F_2N_2O_4Pt \cdot H_2O$: C, H, N.

[Bis(trichloroacetato)][DL-1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II), *meso*-4F-Pt$(Cl_3Ac)_2$: white powder; yield 81%; IR (KBr): 3210 m, 3120 m (NH), 1690 m (C=O), 1610 s, 1240 s, 840 m. *Anal.* Calc. for $C_{18}H_{14}Cl_6F_2N_2O_4Pt \cdot H_2O$: C, H, N.

[*Meso*-1,2-bis(4-fluorophenyl)ethylenediamine][bis(phenylacetato)]platinum(II), *meso*-4F-Pt$(PhAc)_2$: white powder; yield 55%; IR (KBr): 3220 br, 3080 br (NH), 1720 m (C=O), 1520 s, 1460 m, 1240 s, 1170 s, 825 m. *Anal.* Calc. for $C_{30}H_{28}F_2N_2O_4Pt$: C, H, N.

[DL-1,2-Bis(4-fluorophenyl)ethylenediamine][bis(phenylacetato)]platinum(II), *rac*-4F-Pt$(PhAc)_2$: white powder; yield 58%; IR (KBr): 3220 br, 3080 br (NH), 1715 m (C=O), 1520 s, 1460 m, 1240 s, 1180 s, 840 s. *Anal.* Calc. for $C_{30}H_{28}F_2N_2O_4Pt$: C, H, N.

Method B. 0.25 mmol of the respective sulfatoplatinum(II) complex and 0.5 mmol of $Ba(OH)_2$ were dissolved in 60 ml of water and stirred for 24 h. The precipitated $BaSO_4$ was filtered off and the colorless solution was treated with 0.5 mmol of glycolic acid. After stirring for 3 days at 50 °C the solution was lyophilized and the resulting product was recrystallized from methanol/diethyl ether 1:5 (vol./vol.) and air dried.

[*Meso*-1,2-bis(4-fluorophenyl)ethylenediamine]diglycolatoplatinum(II), *meso*-4F-Pt$(OHAc)_2$: white powder; yield 56%; IR (KBr): 3200 m, 3120 m (NH), 1615 m (C=O), 1240 s, 830 m. *Anal.* Calc. for $C_{18}H_{20}F_2N_2O_6Pt$: C, H, N.

[DL-1,2-Bis(4-fluorophenyl)ethylenediamine]diglycolatoplatinum(II), *rac*-4F-Pt$(OHAc)_2$: white powder; yield 34%; IR (KBr): 3200 m, 3100 m (NH), 1620 m (C=O), 1240 s, 840 m. *Anal.* Calc. for $C_{18}H_{20}F_2N_2O_6Pt$: C, H, N.

2.2.4. Kinetic studies, reaction of the platinum complexes with KI

The reaction rate constants of the complexes were determined in deionized water at 37 °C. The experiments were performed in silylanized 1.7 ml glass vials. Stock solutions of the complexes in methanol (0.25 mM) were prepared and diluted 50-fold with aqueous KI solutions of different concentrations (*meso*-4F-$PtCl_2$ and *meso*-4F-Pt$(Ac)_2$: 2.5, 5.0, 7.5 and 10.0 mM, respectively; *meso*-4F-Pt$(ClAc)_2$, *meso*-4F-Pt$(Cl_2Ac)_2$, *meso*-4F-Pt$(Cl_3Ac)_2$, *meso*-4F-Pt$(PhAc)_2$ and *meso*-4F-Pt$(OHAc)_2$: 5.0 mM) to a final concentration of 0.05 mM platinum complex immediately prior to the kinetic experiment. In these solutions the molar I^- surplus amounts to the 50-, 100-, 150- or 200-fold of the complex concentration. The analysis of the product mixture was done in 30 min intervals with HPLC techniques (see Section 2.2.5). Each kinetic experiment was performed 2- to 4-fold. To detect if the differing ionic strength, which results from the increasing KI concentration within the test series, has an influence on the rates of the reaction of *meso*-4F-$PtCl_2$ or *meso*-4F-Pt$(Ac)_2$ with I^-, we performed parallel experiments at a constant ionic strength ($I = 10$ mM, $NaNO_3$).

Table 1
HPLC analyses of 'leaving group' derivatives of *meso*-4F-$PtCl_2$

Compound	t_R (min)	K'	Assignment	Eluting solvent [a]
Meso-4F-$PtCl_2$	7.05	0.81	$Pt(OH_2)_2$	50:50
	7.74	0.98	$PtCl(OH_2)$	
	9.34	1.39	$PtI(OH_2)$	
	10.69	1.74	$PtCl_2$	
	12.98	2.33	PtCl/I	
	17.32	3.44	PtI_2	
Meso-4F-Pt$(Ac)_2$	7.54	1.00	$PtAc(OH_2)$	50:50
	12.79	2.40	$Pt(Ac)_2$	
	14.68	2.91	PtAc/I	
Meso-4F-Pt$(ClAc)_2$ [b]	13.13	3.04	$Pt(ClAc)_2$	55:45
Meso-4F-Pt$(Cl_2Ac)_2$ [b]	16.68	4.18	$Pt(Cl_2Ac)_2$	60:40
Meso-4F-Pt$(Cl_3Ac)_2$	19.88	4.34	$Pt(Cl_3Ac)_2$	70:30
	7.77	1.45	$Pt(Cl_3Ac)/I$	
Meso-4F-Pt$(PhAc)_2$ [b]	26.83	5.88	$Pt(PhAc)_2$	65:35
Meso-4F-Pt$(OHAc)_2$	9.19	1.88	$Pt(OHAc)_2$	50:50
	12.20	2.78	Pt(OHAc)/I	

[a] Isocratic system consisting of a mixture of methanol and 0.001 N H_2SO_4 (20 mM Na_2SO_4).
[b] The intermediates (PtAc/I derivatives) could not be detected.

2.2.5. HPLC analysis of the reaction mixture

The HPLC analyses were done with a Kontron high pressure mixing gradient system (Kontron 430 HPLC pump), a Kontron HPLC autosampler, and a Kontron 430 HPLC UV detector. A 0.4 × 25 cm Nucleosil-100 RP 18 column (Macherey-Nagel, Düren, Germany) with a 0.4 × 3.0 cm precolumn was used for chromatography. After being loaded onto the column, the sample (50 μl) was eluted at r.t. and at a flow rate of 0.6 ml/min with an isocratic system consisting of a mixture (vol./vol., see Table 1) of CH_3OH and of an aqueous solution of H_2SO_4 (0.001 N) and Na_2SO_4 (0.02 M). The void volume elution time (t_0) was 4.0 min. At the used 50- to 200-fold molar I^- surplus only the compounds *meso*-4F-PtX_2, *meso*-4F-PtXI and *meso*-4F-PtI_2 (X = Cl, Ac or Ac-derivatives) could be detected. The assignment of the peaks in the chromatograms to these compounds was performed on the example of the *meso*-4F-$PtCl_2$ reaction by use of reference substances. For identification of the diacetatoplatinum(II) derivatives the $-PtX_2$ and $-PtI_2$ reference substances were at hand.

The starting and reaction products are well detectable by UV spectroscopy. Due to the large extinction coefficient of the diamine ligand at $\lambda_{max} = 268$ nm the sensitivity of the HPLC method is very high. The change in the UV absorption associated with the exchange of the 'leaving groups' is comparatively small, so the uncorrected HPLC data of educt and products are used to determine the rate constants. From each experiment three parallel estimations were performed. Integration of the peak area was done by use of the Kontron 450 MT data system. The mole fractions were calculated from the

respective peak areas as fractions of the total peak area. The rate constants were computed according to the kinetic scheme described in the text by methods of the formal reaction kinetics [18].

2.2.6. Evaluation of the water solubility

The solubility of the new complexes was evaluated by 30 min ultrasonation of a suspension of the respective compound in 10 ml H_2O at r.t., filtration of the saturated solution, freeze drying and weighing of the residue.

2.3. Biological methods

2.3.1. In vitro chemosensitivity assay on the human MCF-7 breast cancer cell line

The in vitro testing of the platinum complexes on antitumor activity was carried out on exponentially dividing human MCF-7 breast cancer cells (American Type Culture Collection, Rockville, MD, USA) according to a previously published microtiter assay [11]. Briefly, in 96-well microtiter plates (Costar), 100 μl of a cell suspension at 500 cells/ml culture medium (Eagle's MEM with 10% fetal calf serum) were plated into each well and incubated at 37 °C for 2–3 days in a humidified atmosphere (5% CO_2). By addition of an adequate volume of a stock solution of the respective compound (solvent: DMF) to the medium the desired test concentration was obtained. For each test concentration and for the control, which contained the corresponding amount of DMF, 16 wells were used. After the proper incubation time the medium was removed, the cells were fixed with a glutardialdehyde solution and stored at 4 °C. Cell biomass was determined by a crystal violet staining technique as described in Ref. [11]. The effectiveness of the complexes is expressed as corrected T/C values according to the following equation: $T/C_{corr} = (T - C_0)/(C - C_0) \times 100$, where T (test) and C (control) are the optical densities at 578 nm of the crystal violet extract of the cell lawn in the wells (i.e. the chromatin-bound crystal violet extracted with ethanol 70%), and C_0 is the density of the cell extract immediately before treatment. For the automatic estimation of the optical density of the crystal violet extract in the wells a Microplate EL 309 Autoreader was used.

2.3.2. In vivo testing on the hormone-insensitive MXT-Ovex mammary carcinoma of the B6D2F1 mouse

The method applied was identical to that described in Ref. [15]. In female B6D2F1 mice (Charles River Wiga, Germany, age: 8 weeks at the beginning of the test, body weight about 20 g) the tumor was transplanted subcutaneously as pieces of about 2 mm^3 (one piece/mouse). The test animals were randomly assigned to groups of ten animals each. On the first day after transplantation, treatment with the drugs (*rac*-4F-$PtCl_2$, *rac*-4F-$PtSO_4$ and *rac*-4F-Pt$(Ac)_2$: 20 μmol/kg; cisplatin: 5 μmol/kg), dissolved in 0.1 ml PEG 400/water 1:1 (vol./vol.) was started. Three times a week (Monday, Wednesday, Friday) 0.1 ml/mouse was injected s.c. for 6 weeks. On day 36, the animals were killed by cervical dislocation and weighed. The tumors were removed and, after washing in 0.9% NaCl solution, were dabbed dry and weighed. Then the median tumor weight was calculated.

3. Results

3.1. Synthesis and spectroscopic characterization

For the synthesis of the [1,2-bis(4-fluorophenyl)-ethylenediamine]platinum(II) complexes with acetic acid and acetic acid derivatives as 'leaving groups', the aqua[1,2-bis(4-fluorophenyl)ethylenediamine]sulfatoplatinum(II) diastereoisomers, *meso*- and *rac*-4F-$PtSO_4$ [17], were used as educts. *Meso*- and *rac*-4F-$PtSO_4$ reacted with acetic acid and its derivatives (Scheme 1, Method A) in quantitative yields and the resulting complexes (*meso*/*rac*-4F-Pt$(Ac)_2$, *meso*/*rac*-4F-Pt$(ClAc)_2$, *meso*/*rac*-4F-Pt$(Cl_2Ac)_2$, *meso*/*rac*-4F-Pt$(Cl_3Ac)_2$, *meso*/*rac*-4F-Pt$(PhAc)_2$) precipitated from the aqueous solution in excellent purity. The highly water soluble complexes *meso*- and *rac*-4F-Pt$(OHAc)_2$ were obtained by reaction of *meso*- and *rac*-4F-Pt$(OH)_2$ with glycolic acid in water (Scheme 1, Method B). The isolation of these products was performed by freeze drying. The elemental analyses, IR and ^1H NMR spectra as well as the HPLC chromatograms revealed that all complexes were free of side products (see Table 2 and Section 2). The educts, *meso*- and *rac*-4F-Pt$(OH)_2$, were synthesized from their sulfatoplatinum(II) analogues in aqueous solution by addition of $Ba(OH)_2$ and subsequent removal of the precipitated $BaSO_4$.

Corresponding to the elemental analyses the complexes contain two carboxylic acid residues. The question whether the complexes exist as diacetato[ethylenediamine]platinum(II) (4F-Pt$(RAc)_2$) or as acetato[ethylenediamine]-platinum(II) acetate ([4F-Pt(RAc)]$^+RAc^-$, R = Cl, Cl_2, Cl_3, OH or Ph) was decided by the differing C=O stretching vibrations of the coordinated and of the free (i.e. ionic) acetic acid ligand. With the exception of *meso*- and *rac*-4F-Pt$(PhAc)_2$ the IR spectra of the complexes exhibit broad bands at 1610–1690 cm^{-1}, consistent with the presence of unidentate acetate ligands [19]. The IR spectra of *meso*- and *rac*-4F-Pt$(PhAc)_2$ show the stretching vibration of a free carboxylato group at $\nu(C{=}O) \approx 1720$ cm^{-1} and additional bands at $\nu(C{=}O) = 1520$ and 1460 cm^{-1}, which are very similar to those of the bidentate coordinated acetate in $Pt(NH_3)_2(Ac)(ClO_4) \cdot H_2O$ [19].

The ^1H NMR spectra of the new complexes were recorded in [D_7]-DMF as solvent. When [D_6]-DMSO was used as solvent one of the two 'leaving' groups of the diacetatoplatinum(II) complexes was exchanged and an asymmetrical PtL(DMSO) moiety (L = Ac and others) was formed. The vicinity to asymmetric C atoms gives rise to a diastereotopical splitting of the NH and the $CH_{benzylic}$ signals. Addition of D_2O to the complex solution led to an NH/ND exchange and to the appearance of an AB system for the benzylic protons.

Table 2
^{1}H NMR data [a] of [1,2-bis(4-fluorophenyl)ethylenediamine]diacetatoplatin(II) complexes

Compound	$CH_{benzylic}$	NH^1	NH^2	Ar–H	Ligand–H
Meso-4F-Pt(Ac)$_2$	4.55 (m, 2H)	6.01 (br, 2H)	6.74 (br, 2H)	7.08–7.15 (m, 4H) 7.59–7.64 (m, 4H)	1.75 (s, 6H, CH_3)
Rac-4F-Pt(Ac)$_2$	4.84 (m, 2H)	6.02 (br, 2H)	7.20 (br, 2H)	7.08–7.15 (m, 4H) 7.66–7.71 (m, 4H)	1.73 (s, 6H, CH_3)
Meso-4F-Pt(ClAc)$_2$	4.68 (m, 2H)	6.14 (br, 2H)	6.86 (br, 2H)	7.06–7.16 (m, 4H) 7.59–7.67 (m, 4H)	3.98 (s, 4H, CH_2)
Rac-4F-Pt(ClAc)$_2$	4.67 (m, 2H)	6.04 (br, 2H)	7.02 (br, 2H)	7.07–7.14 (m, 4H) 7.61–7.67 (m, 4H)	4.01 (s, 4H, CH_2)
Meso-4F-Pt(Cl_2Ac)$_2$	4.46 (m, 2H)	6.17 (br, 2H)	6.84 (br, 2H)	7.10–7.17 (m, 4H) 7.61–7.66 (m, 4H)	6.30 (s, 2H, CH)
Rac-4F-Pt(Cl_2Ac)$_2$	4.49 (m, 2H)	6.09 (br, 2H)	7.00 (br, 2H)	7.07–7.14 (m, 4H) 7.61–7.67 (m, 4H)	6.24 (s, 2H, CH)
Meso-4F-Pt(Cl_3Ac)$_2$	4.47 (m, 2H)	6.31 (br, 2H)	6.97 (br, 2H)	7.11–7.18 (m, 4H) 7.64–7.70 (m, 4H)	
Rac-4F-Pt(Cl_3Ac)$_2$	4.48 (m, 2H)	6.21 (br, 2H)	7.07 (br, 2H)	7.07–7.15 (m, 4H) 7.63–7.68 (m, 4H)	
Meso-4F-Pt(OHAc)$_2$	4.60 (m, 2H)	6.11 (br, 2H)	6.88 (br, 2H)	7.09–7.16 (m, 4H) 7.60–7.66 (m, 4H)	3.78 (m, 6H, CH_2–OH)
Rac-4F-Pt(OHAc)$_2$	4.73 (m, 2H)	6.07 (br, 2H)	7.06 (br, 2H)	7.10–7.16 (m, 4H) 7.61–7.67 (m, 4H)	3.76 (m, 6H, CH_2–OH)
Meso-4F-Pt(PhAc)$_2$	4.40 (m, 2H)	6.03 (br, 2H)	6.64 (br, 2H)	7.05–7.15 (m, 4H) 7.53–7.68 (m, 4H)	3.37 (s, 2H, CH_2) 3.55 (s, 2H, CH_2) 7.06–7.13 (m, 5H, Ph) 7.17–7.34 (m, 5H, Ph)
Rac-4F-Pt(PhAc)$_2$	4.58 (m, 2H)	6.02 (br, 2H)	6.92 (br, 2H)	7.03–7.15 (m, 4H) 7.47–7.55 (m, 4H)	3.36 (s, 2H, CH_2) 3.64 (s, 2H, CH_2) 7.16–7.25 (m, 5H, Ph) 7.27–7.35 (m, 5H, Ph)

[a] The spectra were recorded at 250 MHz as [D_7]-DMF solutions with TMS as internal standard, δ (ppm).

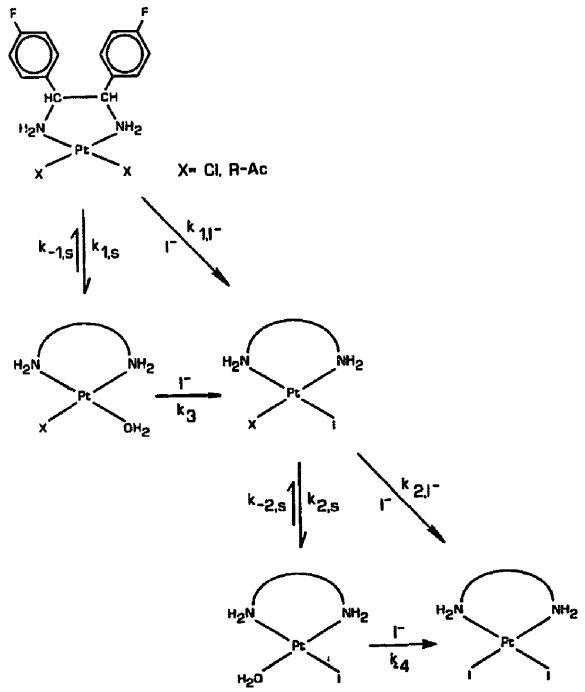
Dependent on the configuration the coupling constants amount to 4.6–5.5 Hz (*meso*-configurated; e.g. *meso*-4F-Pt(Ac)$_2$: δ=4.33, 4.42 ppm, ^3J=5.3 Hz) or 11.5–12.5 Hz (*rac*-configurated; e.g. *rac*-4F-Pt(Ac)$_2$: δ=4.25, 4.33 ppm, ^3J=11.9 Hz). In [D_7]-DMF only one signal was found for the benzylic protons after NH/ND exchange, indicating a symmetrical 4F-PtX_2 molecule (e.g. *meso*-4F-Pt(Ac)$_2$: δ=4.40 ppm; *rac*-4F-Pt(Ac)$_2$: δ=4.66 ppm).

The ^{1}H NMR spectra of *meso*- and *rac*-4F-Pt(PhAc)$_2$ are in accordance with the above proposed structure of a complex containing one bidentate and one ionic phenylacetate. The signals of the free 'leaving group' are located at about δ=3.60 ppm (CH_2) and δ=7.20 ppm (Ar–H), and those of the platinum bound at about δ=3.35 ppm (CH_2) and δ=7.10 ppm (Ar–H) (see Table 2).

3.2. Water solubility of the 'leaving group' derivatives

To find out whether the new 4F-PtX_2 derivatives (X = 'leaving group') are soluble in the concentration range we use in the in vitro and in vivo testing, we performed an approximative gravimetrical estimation of the water solubility yielding the following values: *meso*-4F-PtCl_2 0.3 mg/ml (584 μM); *rac*-4F-PtCl_2 0.2 mg/ml (389 μM); *meso*-4F-Pt(Ac)$_2$ 0.4 mg/ml (736 μM); *rac*-4F-Pt(Ac)$_2$ 0.2 mg/ml (368 μM); *meso*-4F-Pt(ClAc)$_2$ 0.8 mg/ml (1307 μM); *rac*-4F-Pt(ClAc)$_2$ 0.3 mg/ml (490 μM); *meso*-4F-Pt(Cl_2Ac)$_2$ 1.0 mg/ml (1468 μM); *rac*-4F-Pt(Cl_2Ac)$_2$ 0.4 mg/ml (587 μM); *meso*-4F-Pt(Cl_3Ac)$_2$ 1.1 mg/ml (1467 μM); *rac*-4F-Pt(Cl_3Ac)$_2$ 0.5 mg/ml (667 μM); *meso*-4F-Pt(OHAc)$_2$ 4.4 mg/ml (7417 μM); *rac*-4F-Pt(OHAc)$_2$ 4.5 mg/ml (7586 μM); *meso*-4F-Pt(PhAc)$_2$ 0.3 mg/ml (421 μM); *rac*-4F-Pt(PhAc)$_2$ 0.2 mg/ml (280 μM).

These data revealed that the solubility of the complexes was sufficient to achieve cytotoxic drug concentrations (about 10 μM) in cell culture medium as well as in plasma. However, with the exception of the diglycolatoplatinum(II) derivatives, the water solubility of the new complexes was too low for the preparation of injection solutions containing the required drug concentration. The use of drug suspensions in animal experiments often leads to incorrect results, e.g. to inhibition effects markedly weaker than the true antitumor potencies, due to an insufficient bioavailability of the investigated substance. In this case, an exact dose activity relationship cannot be determined. For the in vivo testing of the complexes it is indispensable to develop an appropriate formulation for each compound. With *rac*-4F-Pt(Ac)$_2$, whose testing on the MXT-Ovex breast cancer of the mouse is described in this publication, we could obtain an injection solution by use of a mixture of polyethylene glycol 400 and water 1:1 (vol./vol.).



Scheme 2. Mechanism of the reaction of [1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) complexes with iodide in water.

3.3. Solution chemistry

In order to determine the effect of the exchange of the chloride 'leaving groups' in diastereomeric 4F-$PtCl_2$ complexes by acetic acid (Ac) and acetic acid derivatives (ClAc, Cl_2Ac, Cl_3Ac, OHAc and PhAc), respectively, on the mechanism and the rate of the reaction with bionucleophiles, which in turn influences the pharmacokinetic behavior of this complex type, we performed comparative kinetic studies in aqueous solution using I^- as nucleophile. For these reaction kinetic studies it is of use that I^- displaces the Pt coordinated 'leaving groups', especially H_2O and Cl^-, very quickly and forms a stable Pt–I bond, so none of the reaction steps is reversible. The application of I^- as nucleophile instead of nucleophilic plasma components like S-containing amino acids, peptides or proteins, which inactivate platinum(II) complexes by irreversible binding on their way to the tumor, is of considerable advantage from an experimental point of view (it allows a fast and uncomplicated quantitative determination of the products) and gives useful hints as to the pharmacokinetic behavior of the respective platinum(II) complex (compare Section 4.1). We have shown on the example of stereoisomeric dichloro[1,2-bis(2-hydroxyphenyl)ethylenediamine]platinum(II) complexes that such kinetic studies using I^- as nucleophile contribute to the elucidation of the influence of the neutral ligand on the reactivity of the central platinum atom with nucleophiles, and of the consequences for the antitumor activity resulting therefrom [20].

On the example of diacetato[meso-1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) it was clarified if the new 'leaving group' derivatives reacted predominantly in accordance with the 'solvent path' (i.e. the 'indirect nucleophilic substitution path'; Scheme 2) and if the respective rate constants ($k_{1,s}$ and $k_{2,s}$) were similar to those of cisplatin ($k_{1,s} = 6.32 \times 10^{-5}\,s^{-1}$ and $k_{2,s} = 2.50 \times 10^{-5}\,s^{-1}$ [20]). We assume that such a reaction kinetic behavior is a prerequisite for optimal pharmacokinetic and cytotoxic properties. The studies were performed exclusively with the *R/S*-configurated complexes, which were better soluble in hydrophilic solvents than their *RR/SS*-configurated counterparts. The low solubility of the racemic complexes and of their reaction products prohibited also the quantitative determination of the single components by HPLC. Since there is hardly a difference between the hydrolysis rates of *meso*- and *rac*-4F-$PtCl_2$ ($k_{1,obs}$ (10^{-4} s^{-1})): *meso*-form = 1.26; *rac*-form = 1.11 [21]) it can be inferred that the *rac*-4F-PtX_2 complexes (X = variable 'leaving group') and their *meso*-configurated counterparts possess very similar rate constants regarding the pseudo-first-order as well as the second-order reaction with the nucleophile I^-. Therefore we did not perform kinetic studies in the *RR/SS* series.

The influence of the Cl, Cl_2, Cl_3, OH and Ph substituents in the acetato 'leaving group' on the rate of the reaction with

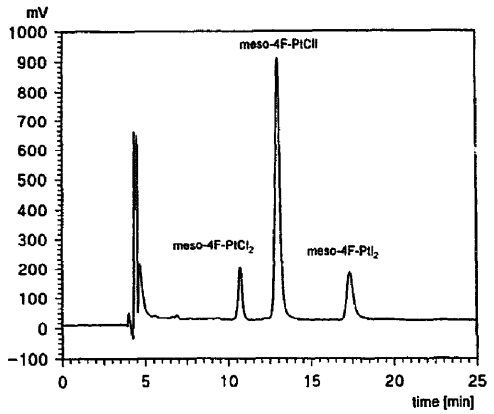


Fig. 1. HPLC chromatogram of the reaction mixture of the reaction of *meso*-4F-$PtCl_2$ (50 μM) with KI (7.5 mM); reaction conditions: solvent: H_2O; reaction temperature: 37 °C; reaction time: 150 min.

nucleophiles was investigated with the same kinetic method (*meso*-4F-PtX_2: 50.0 μM; I^-: 5.0 mM; solvent: H_2O; temperature: 37 °C) by determination of the $k_{1,obs}$ and $k_{2,obs}$ values. These parameters allow the comparison of the 'leaving group' derivatives with respect to their reactivity with nucleophiles, but they give no evidence of any kind as to which one of the two reaction ways, the 'indirect' or the 'direct' nucleophilic substitution path, is dominant.

To monitor the formation rates of the reaction products, an HPLC system with UV detection was used. The educts and the resulting products, *meso*-4F-PtX_2, *meso*-4F-PtXI and *meso*-4F-PtI_2, eluate quantitatively from the RP-18 HPLC column with an excellent separation (compare Scheme 2 and Fig. 1).

3.3.1. Kinetic studies on the reaction of dichloro[meso-1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) and of its diacetatoplatinum(II) derivative with iodide

According to Scheme 2 the substitution of the 'leaving groups' X in *meso*-4F-PtX_2 by a nucleophile can proceed via two different pathways. The first is called the 'solvent path' or the 'indirect nucleophilic substitution path', in which two slow reaction steps, the substitution of a 'leaving group' by a water molecule under formation of a monoaquaplatinum(II) species (hydrolysis, rate constants: $k_{1,s}$, $k_{2,s}$), alternate with two fast reaction steps, the replacement of the water molecule by the nucleophile ($k_3 \gg k_{1,s}$, $k_4 \gg k_{2,s}$). Parallel to this, a direct attack of the nucleophile can occur (rate constants: $k_{1,y}$, $k_{2,y}$, y = nucleophile) in a second nucleophilic substitution pathway (i.e. the 'direct nucleophilic substitution path').

The rate law governing the substitution in planar platinum complexes usually consists of two terms, one first order regarding the metal complex and the other first order regarding both complex and entering nucleophile (y; Eq. (1)).

$$v = k_s[\text{complex}] + k_y[\text{complex}][y] \quad (1)$$

If the nucleophile is used in excess as in the experiments described in this publication, the reaction follows pseudo-first-order dependence. The experimental first-order rate constant (k_{obs}) is described by Eq. (2).

$$k_{obs} = k_s + k_y[y] \quad (2)$$

$k_{1,obs}$ and $k_{2,obs}$ are the observed rate constants for the loss of *meso*-4F-PtX_2 and of *meso*-4F-PtXI, respectively.

Under the employed reaction conditions (50- to 200-fold molar I^- surplus) aquated intermediates (=$Pt(X)(OH_2)^+$, =$Pt(OH_2)_2^{2+}$, X = Cl, I or Ac) are not detectable. However, at an only 2-fold molar I^- surplus, we found the monoaquated intermediates (*meso*-4F-$Pt(X)(OH_2)^+$, X = Cl, I or Ac, respectively; maximum portion about 20% of the educt/product mixture (100%)), but not the =$Pt(OH)_2^{2+}$ species. Fig. 2 shows the typical time dependent concentration change of the educt *meso*-4F-PtX_2, of the intermediate product *meso*-4F-PtXI and of the end product *meso*-4F-PtI_2 on the example of *meso*-4F-$PtCl_2$ (50 μM), which was reacted with a 150-fold molar I^- surplus.

The evaluation of the curves from the reaction of *meso*-4F-$PtCl_2$ and *meso*-4F-$Pt(Ac)_2$ (50 μM) with increasing amounts of the nucleophile (50- to 200-fold molar excess) yields $k_{1,obs}$ and $k_{2,obs}$ values dependent on the concentration of the entering nucleophile. In accordance with the proposed two-path mechanism (compare Scheme 2 and Eq. (2)), the exchange of the 'leaving groups' (Cl and Ac, respectively) gives plots, $k_{1,obs}$ versus $[I^-]$ and $k_{2,obs}$ versus $[I^-]$, which have linear correlations with intercepts $k_{1,s}$ and $k_{2,s}$ and slopes $k_{1,I}$ and $k_{2,I}$ (Fig. 3).

In the reaction of *meso*-4F-$Pt(Ac)_2$ with I^- k_{obs} is independent of the iodide concentration, which suggests that the 'solvent path' is taken preferably. With *meso*-4F-$PtCl_2$, the dependence of k_{obs} on the iodide concentration shows the involvement of both the indirect and direct nucleophilic pathways.

The $k_{1,obs}$ and $k_{2,obs}$ values of *meso*-4F-$PtCl_2$ and *meso*-4F-$Pt(Ac)_2$ and the $k_{1,s}$, $k_{2,s}$, $k_{1,I}$ and $k_{2,I}$ values derived therefrom

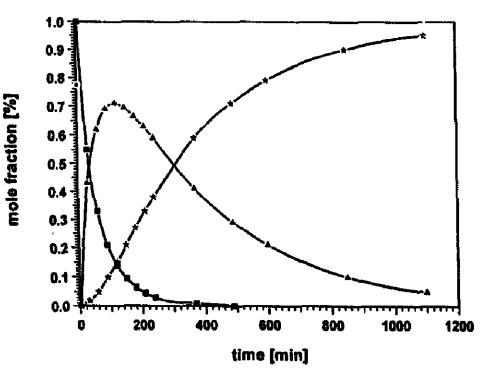


Fig. 2. Reaction time curve of the reaction of *meso*-4F-$PtCl_2$ (50 μM) with KI (7.5 mM); reaction conditions see Fig. 1 (*meso*-4F-$PtCl_2$: ■; *meso*-4F-PtCII: ▲; *meso*-4F-PtI_2: ★).

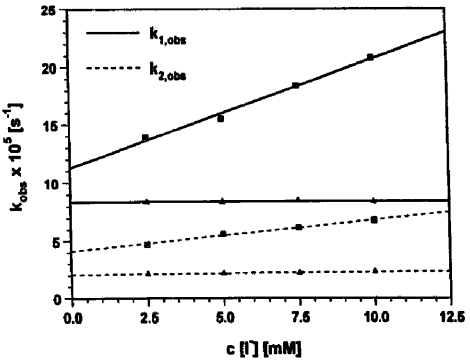


Fig. 3. Reaction rates of *meso*-4F-$PtCl_2$ (■) and *meso*-4F-$Pt(Ac)_2$ (▲) in water at 37 °C as a function of different KI concentrations.

are listed in Table 3. It is of interest to note that the reaction rates were found to be independent of the ionic strength (Table 3, compare the $k_{1,obs}$ and $k_{2,obs}$ values estimated at variable ionic strengths with those estimated at an ionic strength of 10 mM; see also Section 2). During the substitution reactions the pH values of the solutions did not change markedly and amounted to about 6.

The experimental results show that in comparison to *meso*-4F-$PtCl_2$ ($k_{1,s} = 11.3 \times 10^{-5}$ s^{-1}) the hydrolysis rate of *meso*-4F-$Pt(Ac)_2$ ($k_{1,s} = 8.4 \times 10^{-5}$ s^{-1}) is somewhat reduced and is similar to that of cisplatin ($k_{1,s} = 8.0 \times 10^{-5}$ s^{-1} [22]). The $k_{1,I}$ and $k_{2,I}$ values of *meso*-4F-$Pt(Ac)_2$ are much lower than those of *meso*-4F-$PtCl_2$ (Table 3).

3.3.2. The influence of α-substituents in the acetate 'leaving groups' of diacetato[meso-1,2-bis(4-fluorophenyl)ethylene diamine]platinum(II) on the rate of the reaction with iodide

In order to find out how the exchange of acetate by mono-, di- or trichloroacetate (i.e. *meso*-4F-$Pt(ClAc)_2$, *meso*-4F-$Pt(Cl_2Ac)_2$ and *meso*-4F-$Pt(Cl_3Ac)_2$), glycolate (i.e. *meso*-4F-$Pt(OHAc)_2$) and phenylacetate (i.e. *meso*-4F-$Pt(PhAc)_2$) influences the reactivity with bionucleophiles, we determined the $k_{1,obs}$ and $k_{2,obs}$ values of these derivatives using the nucleophile I^- in a concentration of 5.0 mM (Table 4).

Only *meso*-4F-$Pt(ClAc)_2$ proved to be similarly reactive like its parent diacetatoplatinum(II) complex. The $k_{1,obs}$ value of *meso*-4F-$Pt(PhAc)_2$ was comparable to that of carboplatin (Table 4). Presumably the chelated phenylacetate 'leaving group' is responsible for the strongly reduced reactivity of *meso*-4F-$Pt(PhAc)_2$. All other compounds reacted very fast with I^-. The results of these studies show that the reaction kinetic behavior of *meso*- and *rac*-4F-$Pt(Ac)_2$ as well as of *meso*- and *rac*-4F-$Pt(ClAc)_2$ comes close to that which we assume to be a prerequisite for a strong antitumor potency: a moderate hydrolysis behavior comparable with that of cisplatin combined with a resistance against a direct substitution by bionucleophiles (see Section 4.1).

3.4. Tumor inhibiting properties

3.4.1. Human MCF-7 breast cancer cell line

The testing of the 'leaving group' derivatives of the diastereomeric 4F-$PtCl_2$ complexes was performed on the human MCF-7 breast cancer cell line. The test details are described in Section 2. The antitumor effects of the new complexes were compared with those of the parent compounds *meso*- and *rac*-4F-$PtCl_2$ as well as with those of the standards cisplatin and carboplatin. The four reference compounds inhibit the growth of the MCF-7 cell line in a concentration dependent manner (Fig. 4). *Meso*-4F-$PtCl_2$ proved to be similarly active like the first standard cisplatin and much more active than the second standard carboplatin. *Rac*-4F-$PtCl_2$ was even significantly more active than *meso*-4F-$PtCl_2$ and cisplatin (Fig. 4). The exchange of chloride in *meso*-4F-$PtCl_2$ by acetate led to somewhat less active compounds (compare Fig. 4 with Fig. 5, Table 5). The same is true for the replacement of chloride by chloroacetate in *rac*-4F-$PtCl_2$. However, in the case of *meso*-4F-$PtCl_2$ the exchange of chloride by the chloroacetate residue was not accompanied by a decline in antitumor activity (compare Fig. 4 with Fig. 5, Table 5). From the diastereomeric acetato- and chloroacetatoplatinum(II) derivatives, which correspond with cisplatin in their hydrolysis behavior, only *meso*-4F-$Pt(ClAc)_2$ equals the activity of the respective dichloroplatinum(II) complex (compare Fig. 4 with Fig. 5, Table 5). From these results it can be concluded that only *meso*-4F-$Pt(ClAc)_2$ is enriched in the MCF-7 breast cancer cells and bonded to the DNA to a similar extent as its parent compound *meso*-4F-$PtCl_2$. Simultaneously the somewhat lower antitumor activities of *meso*- and *rac*-4F-$Pt(Ac)_2$ and of *rac*-4F-$Pt(ClAc)_2$ point to a reduced intracellular level of these drugs compared to that of the diastereomeric dichloroplatinum(II) analogues. *Meso*- and *rac*-4F-$Pt(PhAc)_2$, whose hydrolysis rates are comparable to that of carboplatin, produce a 50% inhibition of the proliferation of MCF-7 cells in 5 μM concentration, while carboplatin shows the same effect only in a 10 μM concentration (Table 5). From the 'leaving group' derivatives which are under physiological conditions faster hydrolyzed than cisplatin (i.e. 4F-$Pt(Cl_2Ac)_2$, 4F-$Pt(Cl_3Ac)_2$, 4F-$Pt(OHAc)_2$), only the 4F-$Pt(OHAc)_2$ diastereoisomers produce effects comparable to those of their respective dichloroplatinum(II) complexes (Table 5, Fig. 5). *Rac*-4F-$Pt(OHAc)_2$ even proved to be equipotent to cisplatin.

3.4.2. MXT-Ovex breast cancer of the B6D2F1 mouse

In Section 3.3 we have shown that only the diastereomeric 4F-$Pt(Ac)_2$ complexes and their 4F-$Pt(ClAc)_2$ analogues possess the kinetic behavior in the reaction with the nucleophile I^- which we assume to be a prerequisite for the formation of free drug levels permitting a maximal antitumor potency (for details see Section 4.1). However, the drug level is not only dependent on an appropriate reaction kinetics but also on a good bioavailability, which in turn requires a sufficient water solubility of the drug. To find out whether

Table 3
Reaction rate constants [a] of *meso*-4F-$PtCl_2$ and *meso*-4F-$Pt(Ac)_2$

[I] (mM)	I [c] (mM)	*Meso*-4F-$PtCl_2$ [b]		*Meso*-4F-$Pt(Ac)_2$ [b]	
		$k_{1,obs}\times10^5$ (s^{-1})	$k_{2,obs}\times10^5$ (s^{-1})	$k_{1,obs}\times10^5$ (s^{-1})	$k_{2,obs}\times10^5$ (s^{-1})
2.50	2.50	13.9±0.1	4.7±0.2	8.4±0.4	2.1±0.4
5.00	5.00	15.5±0.1	5.6±0.4	8.4±0.3	2.2±0.3
7.50	7.50	18.4±0.4	6.1±0.4	8.5±0.7	2.2±0.1
10.00	10.00	20.8±0.2	6.8±0.6	8.4±0.1	2.3±0.5
2.50	10.00	13.6±0.3	4.5±0.3	8.3±0.3	1.9±0.5
5.00	10.00	15.8±0.4	4.9±0.3	8.6±0.4	1.8±0.3
7.50	10.00	17.5±0.2	6.0±0.1	8.3±0.3	2.1±0.4
10.00	10.00	20.8±0.6	6.6±0.2	8.3±0.2	2.0±0.3
	I [c] (mM)	$k_{1,s}\times10^5$ (s^{-1})	$k_{1,l}\times10^5$ ($l\,mol^{-1}\,s^{-1}$)	$k_{2,s}\times10^5$ (s^{-1})	$k_{2,l}\times10^5$ ($l\,mol^{-1}\,s^{-1}$)
Meso-4F-$PtCl_2$		11.3±0.1	943±20	4.2±0.2	267±10
	10	11.1±0.1	932±30	3.7±0.2	294±8
Meso-4F-$Pt(Ac)_2$		8.4±0.1	5.2±0.4	2.1±0.3	20±0.3
	10	8.5±0.1	12.0±0.2	1.8±0.1	28±0.1

[a] Mean ± standard error.
[b] The platinum complexes (50 μM) were reacted with I^- as nucleophile in water at 37 °C.
[c] Ionic strength.

Table 4
Reaction rate constants [a] of *meso*-4F-$PtCl_2$ and its 'leaving group' derivatives

Compound [b]	$k_{1,obs}\times10^5$ (s^{-1})	$k_{2,obs}\times10^5$ (s^{-1})
Meso-4F-$PtCl_2$	15.9±0.1	5.6±0.4
Meso-4F-$Pt(Ac)_2$	9.4±0.3	3.1±0.5
Meso-4F-$Pt(ClAc)_2$	5.7±0.2	fast
Meso-4F-$Pt(Cl_2Ac)_2$	fast	fast
Meso-4F-$Pt(Cl_3Ac)_2$	51.9±0.7	105.9±0.8
Meso-4F-$Pt(OHAc)_2$	40.8±0.4	5.6±0.5
Meso-4F-$Pt(PhAc)_2$	0.93±0.04	fast
Carboplatin	0.70±0.03	0.80±0.03

[a] The platinum complexes (50.0 μM) were reacted with I^- (5.00 mM) in water at 37 °C.
[b] Mean ± standard error.

4F-$Pt(Ac)_2$ and 4F-$Pt(ClAc)_2$ complexes can produce significant antitumor effects in vivo, we performed a preliminary study on the hormone insensitive, murine MXT-Ovex breast cancer using *rac*-4F-$Pt(Ac)_2$ as model substance and *rac*-4F-$PtCl_2$, *rac*-4F-$PtSO_4$ and cisplatin as comparative substances. The compounds were administered equimolarly in the dosage 20 μmol/kg s.c., three times per week; cisplatin was used as positive control in the subtoxic dosage of 5 μmol/kg. The test data are shown in Fig. 6. As expected *rac*-4F-$PtCl_2$ proved to be inactive on the MXT-Ovex breast cancer in our study as a result of an inadequate bioavailability. In contrast to this the water soluble *rac*-4F-$PtSO_4$ markedly inhibited the growth of the MXT-Ovex mammary tumor. We assume that after the administration of this compound its dichloroplatinum(II) derivative is quickly formed ($t_{1/2}$ concerning the reaction in 0.9% NaCl is about 2 min), and by this an effective drug level similar to that after administration of the equivalent dose of a *rac*-4F-$PtCl_2$ hydrosol preparation is achieved (compare the effects of *rac*-4F-$PtSO_4$ and *rac*-4F-$PtCl_2$ hydrosol in Fig. 6). In our experiments the antitumor activity of *rac*-4F-$Pt(Ac)_2$ matched that of the active standards. This shows that the changed reaction behavior resulting from the substitution of the 'leaving group' chloride in 4F-$PtCl_2$ by acetate indeed leads to an amelioration of the pharmacokinetic properties. In comparison to *rac*-4F-$Pt(Ac)_2$ cisplatin proved to be somewhat more active (Fig. 6).

4. Discussion of the relationship between chemical reactivity and biological activity

4.1. The influence of the 'leaving groups' on the reaction mechanistic behavior

In this work we attempted to ameliorate the inadequate pharmacokinetics of diastereomeric 4F-$PtCl_2$ complexes by exchange of the chloride 'leaving groups' by more stably bound carboxylic acids. Analogous studies with cisplatin had shown that a marked increase of the free drug level (i.e. the non-plasma protein bound part of the drug) could be achieved by this measure. An example is carboplatin, which contains cyclobutane-1,1-dicarboxylate as 'leaving group'. Since the hydrolysis rate constants of carboplatin are so much smaller than those of cisplatin (e.g. $k_{1,s}$ of carboplatin = 7.2×10^{-7} s^{-1}; $k_{1,s}$ of cisplatin = 8.0×10^{-5} s^{-1} [22]), the improve-

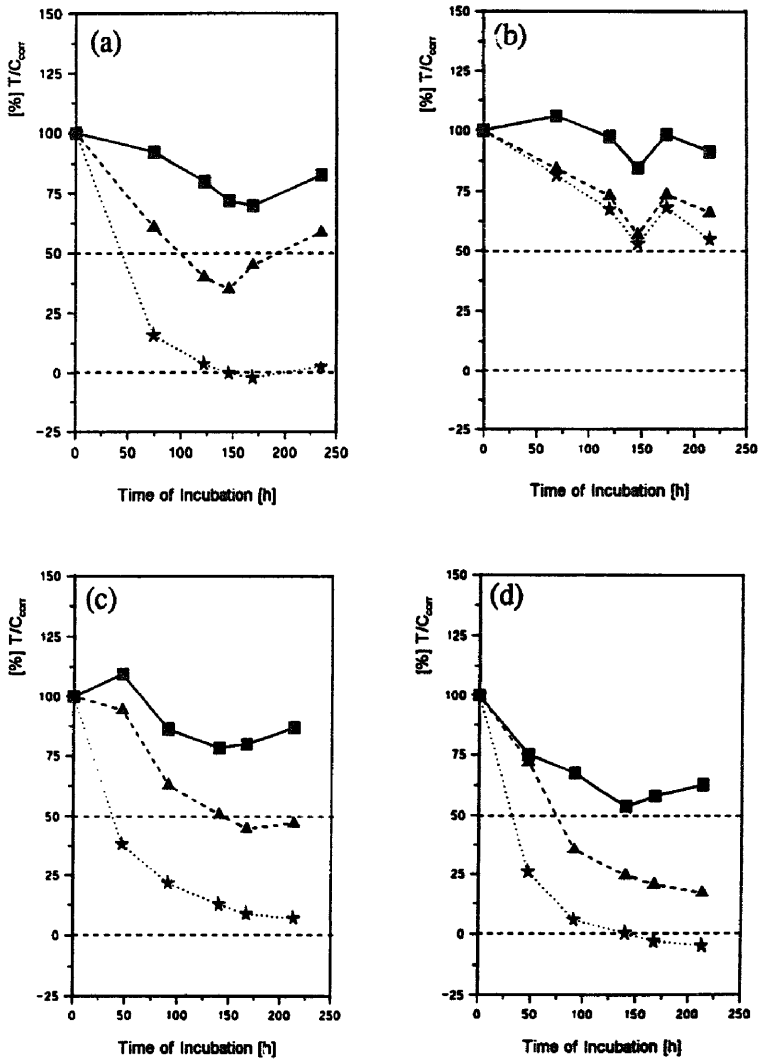


Fig. 4. Effect of cisplatin (a), carboplatin (b), *meso*-4F-$PtCl_2$ (c) and *rac*-4F-$PtCl_2$ (d) on the MCF_7 mammary cell line at the concentrations of 0.5 (■), 1 (▲) and 5 (★) μM.

ment of the pharmacokinetic behavior is gained at the expense of a considerable decrease in its antitumor potency (compare Table 5 and Section 1). Therefore we have searched for 'leaving groups' (X) other than cyclobutane-1,1-dicarboxylate, which endow the diastereomeric 4F-PtX_2 complexes with the following reaction mechanistic properties that are optimal for their pharmacokinetic behavior as well as for their cytotoxicity:

(i) reaction rate constants in the 'solvent path' ($k_{1,s}$, $k_{2,s}$) which are comparable with those of cisplatin;

(ii) the prevalence of the indirect nucleophilic substitution path.

In this context it is important to know that only the aquated species of platinum(II) complexes can react with the nucleobases of DNA [3]. Therefore the rate constants $k_{1,s}$ and $k_{2,s}$ indicate how fast the irreversible binding of the platinum complex to DNA and the onset of the cytotoxic effect take place. They also suggest the rate of the complex inactivation by bionucleophiles via the 'solvent path' (i.e. the 'indirect nucleophilic substitution path'). The absence of a direct replacement of the 'leaving groups' by bionucleophiles, i.e. the 'direct nucleophilic substitution path' (Scheme 2), which contributes to the irreversible binding of platinum(II) complexes to bionucleophiles, leads to an increase of the free drug

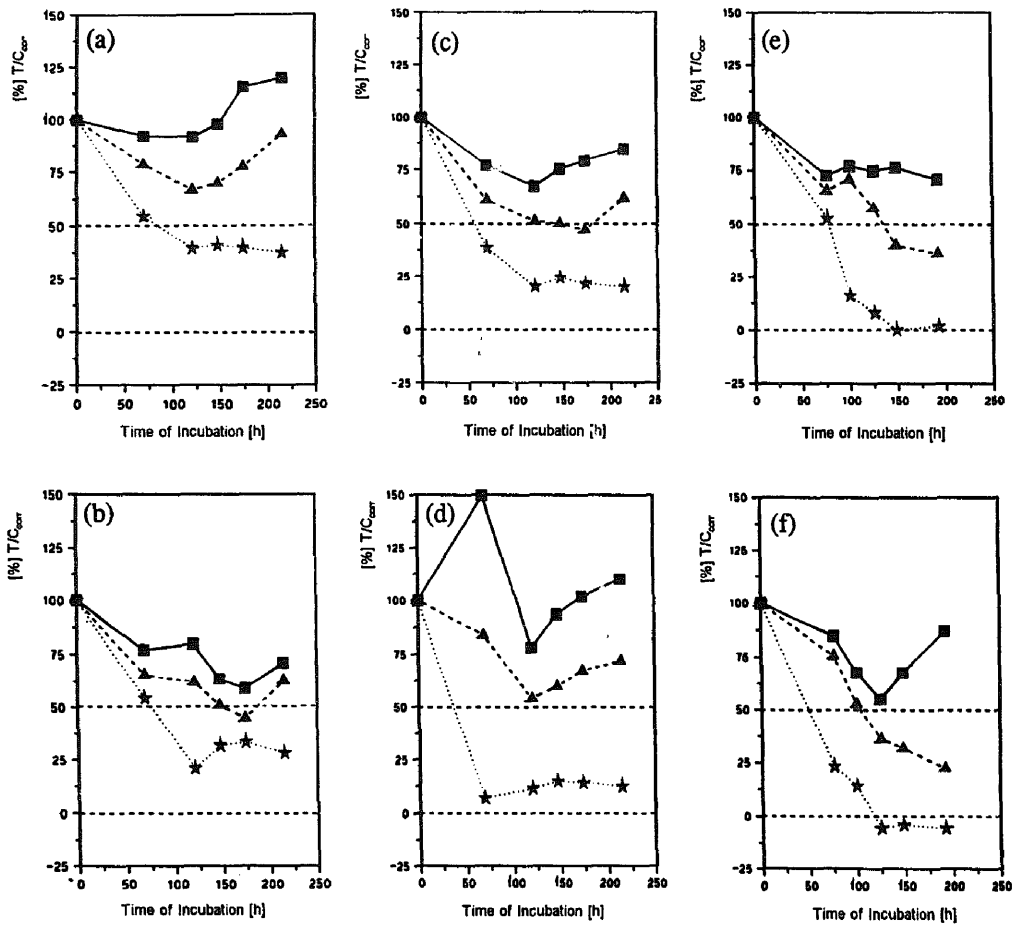


Fig. 5. Effect of *meso*-4F-Pt(Ac)$_2$(a), *rac*-4F-Pt(Ac)$_2$(b), *meso*-4F-Pt(ClAc)$_2$(c), *rac*-4F-Pt(ClAc)$_2$(d), *meso*-4F-Pt(OHAc)$_2$(e) and *rac*-4F-Pt(OHAc)$_2$(f) on the MCF$_7$ mammary cell line at the concentrations of 0.5 (■), 1 (▲) and 5 (★) μM.

level and by this to an improvement of the antitumor potency in vivo. Bionucleophiles which inactivate platinum(II) complexes during the transport to the tumor are mainly plasma components like amino acids, peptides and proteins. The favored binding sites for platinum(II) complexes are the most nucleophilic, S-containing residues of these molecules as shown by structural elucidation of several platinated plasma components [23–30]. For albumin, the major protein in plasma, it has been shown that cisplatin in fact becomes covalently attached to a sulfhydryl group, namely to that of Cys 34 [25]. The existence of two reaction paths for the in vivo inactivation process for platinum(II) complexes is supported by kinetic studies on the reaction of cisplatin with Cys, Homocys, Met and GSH, which were performed under physiological conditions [27–29]. The second-order rate constant of the reaction of cisplatin with Cys, for example, was $k = 386 \times 10^{-4}$ M^{-1} s^{-1} (hydrolysis constant of cisplatin: $k = 1.17 \times 10^{-4}$ s^{-1}) [26]. Dichloro[*meso*-1,2-bis(2,6-dichloro-4-hydroxyphenyl) ethylenediamine]platinum(II) (*meso*-2,6Cl$_2$/4OH-PtCl$_2$), a structure analogue of *meso*-4F-PtCl$_2$, also undergoes a direct as well as an indirect substitution of the chloride 'leaving groups' in the reaction with Cys. Its $k_{1,s}$ and $k_{1,Cys}$ values (0.97×10^{-4} s^{-1} and 234×10^{-4} M^{-1} s^{-1}, respectively, at pH 7.0) are similar to those of cisplatin [23]. The important contribution of the direct substitution reaction in the inactivation of dichloroplatinum(II) complexes by Cys is obvious from the comparison of the loss of *meso*-2,6Cl$_2$/4OH-PtCl$_2$ (10 μM) in water at pH 7.4/37 °C in the absence and in the presence of Cys (10 mM), respectively, which follows pseudo-first-order kinetics (hydrolysis: $k_{obs} = 0.9 \times 10^{-4}$ s^{-1}; Cys reaction: $k_{obs} = 5.0 \times 10^{-4}$ s^{-1}). The fact that at pH 2.8 the k_{obs} value of the Cys reaction (1.2×10^{-4} s^{-1}) is similar to the k_{obs} value of the hydrolysis points to the thiolate form as the active nucleo-

Table 5
Growth inhibition of the human MCF_7 breast cancer cell line by 'leaving group' derivatives of the diastereomeric 4F-$PtCl_2$ complexes

Compound	Approximate %T/C values after 125 h drug-cell-contact at a concentration of (μM)			
	0.5	1.0	5.0	10.0
Cisplatin	80	30	0	
Carboplatin			95	65
Meso-4F-$PtCl_2$	80	50	15	
Rac-4F-$PtCl_2$	73	37	5	
Meso-4F-$Pt(Ac)_2$	90	70	40	
Rac-4F-$Pt(Ac)_2$	75	60	20	
Meso-4F-$Pt(ClAc)_2$	65	50	18	
Rac-4F-$Pt(ClAc)_2$	75	55	12	
Meso-4F-$Pt(Cl_2Ac)_2$	100	78	32	
Rac-4F-$Pt(Cl_2Ac)_2$	87	70	40	
Meso-4F-$Pt(Cl_3Ac)_2$	100	105	73	
Rac-4F-$Pt(Cl_3Ac)_2$	97	77	37	
Meso-4F-$Pt(OHAc)_2$	75	62	8	
Rac-4F-$Pt(OHAc)_2$	55	38	−3	
Meso-4F-$Pt(PhAc)_2$	95	96	67	
Rac-4F-$Pt(PhAc)_2$	120	93	52	
Meso-4F-$PtSO_4$	77	62	12	
Rac-4F-$PtSO_4$	60	32	5	

phile [23]. A direct substitution of the chloride 'leaving groups' in *meso*-2,6Cl_2/4OH-$PtCl_2$ was also shown for other S-containing amino acids [23].

The available data concerning the mechanism of the reaction of dichloroplatinum(II) complexes with plasma, which was studied on the example of cisplatin, are controversal. LeRoy and Thompson [31] have reported that the formation of the aquated cisplatin species is the rate-determining step of this reaction, while the kinetic data of Repta and Long [32] for the reaction of cisplatin in human plasma, plasma fractions and human albumin solutions suggest that the platination of the plasma components takes place indirectly by the hydrolysis product *cis*-aquachlorodiammineplatinum(II) as well as directly by cisplatin itself (for the reaction of *meso*-2,6Cl_2/4OH-$PtCl_2$ with plasma and plasma proteins see Ref. [33]).

Our kinetic experiments on the reaction of *meso*-4F-$PtCl_2$ with I^- have shown that the found pseudo-first-order rate constants correspond to its hydrolysis rate constants which were reported by Bednarski and Trümbach [21]. They are comparable with the hydrolysis constants of *meso*-2,6Cl_2/4OH-$PtCl_2$ and cisplatin, respectively, reported by Bednarski [23]. It is of interest that also the second-order rate constants for the substitution of Cl^- by I^- in *meso*-4F-$PtCl_2$ are similar to those of *meso*-2,6Cl_2/4OH-$PtCl_2$ [34] [5] suggesting an analogous reaction behavior of both compounds against plasma components. The fact that the pseudo-first-order and second-order rate constants of dichloro[1,2-diphenylethylenediamine]platinum(II) complexes, which were obtained in the reaction of I^- [34] and S-containing amino acids [23], respectively, are similar [23,34], proves the usefulness of our reaction kinetic method, which uses I^- as nucleophile, as part of a prescreening. It allows the judgement of whether a new platinum(II) complex possesses a reaction kinetic behavior optimal for a therapeutical application [6].

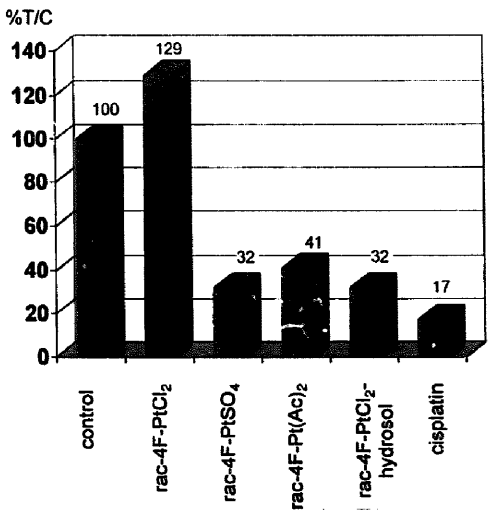


Fig. 6. Effect of platinum complexes on the MXT-Ovex mammary carcinoma of the BDF_1 mouse (dose: 20 μM, solvent: PEG 400/water 1:1 (*rac*-4F-$PtCl_2$, *rac*-4F-$Pt(Ac)_2$) or water (*rac*-4F-$PtSO_4$, *rac*-4F-$PtCl_2$ hydrosol).

[5] Data from Ref. [34]: *m*-2,6Cl_2/4OH-$PtCl_2$: $k_{1,s}=8.82\times10^{-5}$ s^{-1}, $k_{1,l}=608\times10^{-5}$ l/mol s, $k_{2,s}=3.11\times10^{-5}$ s^{-1}, $k_{2,l}=215\times10^{-5}$ l/mol s.

[6] Though the studies revealed a similar kinetic behavior of *meso*-4F-$PtCl_2$ and cisplatin, they did not explain why this complex was bonded to plasma components to a greater extent than cisplatin (compare Section 1). Presumably the lipophilicity of *meso*-4F-$PtCl_2$ plays an important additional role in the irreversible binding to plasma components.

In our studies reported in Section 3.3, *meso*-4F-Pt(Ac)$_2$ has proved to be the 'lead substance' with the desired reaction kinetic behavior (i) and (ii). The $k_{1,s}$ value of the reaction of *meso*-4F-Pt(Ac)$_2$ with I^- is somewhat smaller than that of *meso*-4F-PtCl$_2$ (Table 3) but corresponds with the hydrolysis constant k_1 of cisplatin [22]. However, with *meso*-4F-Pt(Ac)$_2$ the contribution of the direct substitution of the 'leaving groups' by nucleophiles to the total turnover seems to be negligible in comparison to that with cisplatin and *meso*-4F-PtCl$_2$. This is obvious from the identical $k_{1,obs}/k_{1,s}$ and $k_{2,obs}/k_{2,s}$ values, respectively, of *meso*-4F-Pt(Ac)$_2$ (Table 3).

Among the five 'leaving group' analogues derived from 4F-Pt(Ac)$_2$ only 4F-Pt(ClAc)$_2$ possesses a reaction rate similar to that of the parent compound (Table 4). The dichloroacetate and the trichloroacetate residues are weakly bound to platinum and therefore are faster exchanged by bionucleophiles than acetate in the 'lead substance' 4F-Pt(Ac)$_2$. It must be assumed that in vivo these compounds form free drug levels too low to cause significant antitumor effects. It is remarkable that 4F-Pt(PhAc)$_2$ showed a $k_{1,s}$ value like carboplatin, presumably due to its chelate structure (Table 4). The diastereomeric 4F-Pt(OHAc)$_2$ complexes, which hydrolyze markedly faster than cisplatin, too, could be of therapeutical interest owing to their good water solubility warranting a better bioavailability than their poorly soluble 4F-Pt(Ac)$_2$ and 4F-Pt(ClAc)$_2$ analogues. We assume that, after parenteral administration, 4F-Pt(OHAc)$_2$ is more slowly transformed into the dichloroplatinum(II) derivatives (and to a lesser extent into inactive 4F-Pt derivatives of plasma components) than the in vivo active 4F-PtSO$_4$ complexes, whose sulfate 'leaving group' can be more easily exchanged by nucleophiles. Because of these properties the 4F-Pt(OHAc)$_2$ complexes should yield higher free drug levels and in turn should cause stronger antitumor effects in vivo than the 4F-PtSO$_4$ complexes as well as the 4F-PtCl$_2$ hydrosol.

4.2. The influence of the reaction kinetic behavior on the antitumor activity

The influence of the exchange of chloride in *meso*-4F-PtCl$_2$ by the more stably bound acetate on the antitumor activity was determined in a comparative study on the human MCF-7 breast cancer cell line (Table 5). The result of this structural change was a small but significant decline in the antitumor potency of this complex type, which was in accordance with the observation that the hydrolysis rate of *meso*-4F-Pt(Ac)$_2$ is somewhat smaller than that of *meso*-4F-PtCl$_2$. The complex *meso*-4F-Pt(Ac)$_2$ also proved to be less active than cisplatin, though both compounds possessed very similar hydrolysis rate constants. Using the *RR*/*SS*-configurated diamine ligand as educt we obtained a complex (i.e. *rac*-4F-Pt(Ac)$_2$) which produced an effect on the MCF-7 cell line comparable with that of *meso*-4F-PtCl$_2$, but weaker than that of *rac*-4F-PtCl$_2$ and of cisplatin. Since the diastereomeric 4F-Pt(Ac)$_2$ complexes met the reaction kinetic criteria (i) and (ii), we expected an inhibitory effect on the MCF-7 breast cancer cell line stronger than that of *meso*- and *rac*-4F-PtCl$_2$ and also of cisplatin. We explain the weaker antitumor potency of the diastereomeric 4F-Pt(Ac)$_2$ complexes compared to that of cisplatin with a reduced drug uptake by the tumor cell caused by the changed 'leaving group' structure. Conclusions that differences in the antitumor activity of structurally analogous platinum(II) complexes are mainly due to individual uptake kinetics are also supported by studies with mixed-amine cisplatin analogues [35] and with differently ring-substituted dichloro[1,2-diphenylethylenediamine]platinum(II) complexes [21], in which no correlation between hydrolysis rates and cytotoxic potencies was found.

Structural variations in the 'leaving group' of 4F-Pt(Ac)$_2$ like introduction of one to three Cl atoms (i.e. *meso*-4F-Pt(ClAc)$_2$, *meso*-4F-Pt(Cl$_2$Ac)$_2$ and *meso*-4F-Pt(Cl$_3$Ac)$_2$) retained only in the case of the 4F-Pt(ClAc)$_2$ complexes the desired moderate hydrolysis rate of the parent compound. *Meso*- and *rac*-4F-Pt(ClAc)$_2$ showed about the same inhibitory effect on the MCF-7 cell line as *rac*-4F-Pt(Ac)$_2$. The diastereomeric 4F-Pt(Cl$_2$Ac)$_2$ and 4F-Pt(Cl$_3$Ac)$_2$ complexes, whose 'leaving groups' are only loosely bound to platinum, possess a comparable or lower antitumor activity than their parent compounds *meso*- and *rac*-4F-Pt(Ac)$_2$ (Table 5). Therefore they are of minor interest for a further investigation. At 5 μM the diastereomeric 4F-Pt(PhAc)$_2$ complexes, whose $k_{1,s}$ values correspond with that of carboplatin, cause the same effect on the MCF-7 cell line as carboplatin at 10 μM (Table 5). Presumably the chelate structure of the Pt-coordinated phenylacetate (one PhAc acts as counterion) is responsible for the increased hydrolytic stability. However, the solubility of the 4F-Pt(PhAc)$_2$ complexes is insufficient for the preparation of simple aqueous injection solutions appropriate for parenteral administration in patients (see Section 3.2). Further efforts are necessary to resolve this problem, e.g. by design of a galenic formulation similar to the *rac*-4F-PtCl$_2$ hydrosol [4], or by synthesis of a derivative with an additional hydrophilic residue in the 'leaving group'. The same is true for the insufficiently water soluble 4F-Pt(Ac)$_2$ and 4F-Pt(ClAc)$_2$ diastereoisomers. The only compounds of which injection solutions can be produced are the 4F-Pt(OHAc)$_2$ diastereoisomers. Both complexes, whose antitumor potency approaches that of cisplatin (*rac*->*meso*-form), belong to the most active representatives of this 'leaving group' series. However, they do not yet possess the reaction kinetic properties (i) and (ii), which presumably guarantee an optimal antitumor activity in vivo. A water soluble, more hydrolysis resistant carboxylic acid 'leaving group' derivative seems to be available with [*rac*-1,2-bis-(4-fluorophenyl) ethylenediamine] [3-sulfopropionato]platinum(II) (*rac*-4F-Pt(3-HOSO$_2$Prop)), as preliminary experiments have shown. This compound proved to be significantly more active than cisplatin on the human MDA-MB-231 breast cancer cell line [36]. The 'leaving group' derivatives *meso*- and *rac*-4F-Pt(Ac)$_2$, *meso*- and *rac*-4-F-Pt(ClAc)$_2$ as

well as *meso*- and *rac*-4F-Pt(OHAc)$_2$, which at the best produce antitumor effects like cisplatin when used in equimolar concentrations, stand out favorably against carboplatin. In contrast to these 4F-PtX$_2$ complexes carboplatin turns only very slowly into its active aquated derivatives, which makes a markedly higher dosage of carboplatin than of cisplatin and of the new 4F-PtX$_2$ complexes necessary to achieve equal effects.

In a study on the murine MXT-Ovex breast cancer we tried to find out whether 4F-PtX$_2$ complexes endowed with the demanded reaction kinetic properties (i) and (ii) were in fact more effective in vivo than the more reactive comparison compounds *rac*-4F-PtCl$_2$, *rac*-4F-PtSO$_4$ and *rac*-4F-PtCl$_2$ hydrosol. These experiments were performed with *rac*-4F-Pt(Ac)$_2$ [15]. As expected, *rac*-4F-PtCl$_2$, suspended in a polyethylene glycol 400/water 1:1 (vol./vol.) mixture, did not show an inhibition of the tumor growth, presumably due to its insufficient bioavailability (compare Ref. [4]). This surmise is supported by the finding that the exchange of the Cl^- 'leaving groups' by SO_4^{2-} leads to a water soluble, antitumor active derivative (*rac*-4F-PtSO$_4$) [15]. Owing to its high reactivity *rac*-4F-PtSO$_4$ causes strong toxic side effects. We assume that the antitumor activity of *rac*-4F-PtSO$_4$ stems from its dichloroplatinum(II) derivative, which is quickly formed in plasma after injection (compare Section 3.4.2). The disadvantage of *rac*-4F-PtSO$_4$ was overcome by the introduction of the more tightly bound acetate 'leaving group', but only partially, since, at the same time, this group apparently confers a reduced drug uptake by the tumor cell. *Rac*-4F-Pt(Ac)$_2$ was well tolerated, but its antitumor activity did not surpass that of *rac*-4F-PtSO$_4$. We expect that therapeutically more interesting 4F-PtX$_2$ complexes can be developed by use of carboxylic acids containing a hydrophilic residue, e.g. the SO_3H group like in *rac*-4F-Pt(3-HOSO$_2$-Prop) [36], as 'leaving group' (compare Section 4.2). The same considerations apply to diastereomeric 4F-Pt(PhAc)$_2$ complexes, whose reaction kinetic behavior and biological properties compare with those of carboplatin.

5. Conclusions

The test procedure which we evaluated on a series of new diacetatoplatinum(II) compounds permits the preselection of platinum(II) complexes as candidates for expensive in vivo studies. From the novel diacetatoplatinum(II) compounds *meso*/*rac*-4F-Pt(Ac)$_2$, *meso*/*rac*-4F-Pt(ClAc)$_2$, *meso*/*rac*-4F-Pt(OHAc)$_2$ and *meso*/*rac*-4Fn-Pt(PhAc)$_2$ proved to be interesting developmental substances. For a final assessment of the therapeutic merit of these compounds further studies on the pharmacokinetics, the toxicity, and the antitumor activity are planned. They will show whether the substances possess larger therapeutic indices than the standards cisplatin and carboplatin.

Acknowledgements

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